Appendix A

Diversity amongst anti-hapten antibodies

This appendix outlines some of the studies that have been performed to determine the extent of variation between different antibody molecules raised against a common, simple determinant. Four different examples are provided, in which four different haptens were used as the immunogen. Copies of the respective academic publications are provided herewith.

These and other studies demonstrate that widely diverse sequences are generated during the immune response to any particular antigen, due to a combination of V, D, and J region selection, VJ and VDJ splicing, and somatic mutation.

The operation of these events make it essentially impossible to identically reproduce an antibody with a somatically mutated sequence by immunizing a second animal. Clones producing antibody molecules with identical sequences have been described only: a) when based on a nearly *un-mutated germ-line sequence*, in which case the antibody is observable during the primary response; or b) when obtained from the *same* animal, in which case the responsible antibody-producing cells are derived from the same clonal progenitor.

Example 1:

Nahmias et al. (1988, J. Immunol. 140:1304) raised a panel of 14 monoclonal antibodies against the ß-adrenergic hapten alprenolol, also using the same mouse strain. The 14 antibodies utilized at least seven V_L, four J_L, eight V_H, and three J_H genes (Table I), and also demonstrated extensive splicing and mutational diversity (Figures 3-5). Only three pairs of hybridomas used the same H and L chain gene rearrangements; each related pair was obtained from a single mouse and was apparently derived from the same clone (page 1308, ¶ 3).

Example 2:

Stenzel-Poore et al. (1989, J. Immunol. 143:4123) raised a panel of monoclonal antibodies against phosphocholine (PC) in BALB/c mice and F1 hybrids. Fourteen monoclonal antibodies were selected from the high affinity Group II anti-PC that emerge in the secondary

response. The 14 antibodies utilized four V_L, six J_L, six to eleven V_H, four J_H genes, and more than five D genes (Table III). The antibodies each had an average of 3.6 replacement mutations in the heavy chain and 3.1 replacement mutations in the light chain (Table V). Here, as in other studies, the mutations were found *throughout the variable region* – they were enriched in the complementarity determining region (CDR), but also occurred in the framework (Table V).

Example 3:

Blier et al. (1987, J. Immunol. 139:3996) obtained monoclonal antibodies specific for 4-hydroxy-3-nitrophenyl acetate (NP). Twenty eight hybridomas were obtained during the secondary response in the *same mouse*. Fourteen were derived from different clones. Amongst the 14 families, about 3 different V_H, 3 different D and 3 different J_H were used (Table I). Nine families used the same V_H and D region genes, but were all spliced differently to create differences in the CDR4 region (Figure 1). Amongst the 28 antibody panel, there was an average of *8.1 amino acid replacements in each heavy chain variable region* (Table III). On average, *2.5 replacements had occurred after divergence of members of each clone family* (Table III). This indicates that somatic mutation is an *ongoing* process within B-cell clones during the immune response.

Example 4:

Leahy et al. (1988, Proc. Natl. Acad. Sci. USA 85:3661) raised a panel of 12 monoclonal antibodies against a DNP spin-label hapten. The mouse strain used was BALB/c, the same as was used in the disclosure of the instant application to develop 1A7. The amino acid sequences of both the heavy and light chains of the Leahy panel demonstrate that different clones are derived from different germ-line V genes, exhibit junctional diversity around the splice sites, and show mutational divergence from common germ-line precursor sequences. As a result, the sequences are dramatically different amongst the antibodies. These sequences are reproduced below:

 Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 3661–3665, June 1988

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              -15        -5   1          10         20         30  35 36
               |          |   |           |          |          |  AB
ANO2          MRVLILLWLFTAFPGILSDVQLQESGPGLVKPSQSQSLTCTVTGYSITSDYAWN WI
ANO1          .K..S..Y.L..I.......................L....S.........G.Y.. ..
ANO3          .K..S..Y.L..I.......................L....S.........G.Y.. ..
ANO7          .......C..................D.........L..............G.S.H ..
ANO5         ME.HW.F.F..SVTA.VH.QF.P.Q..AE.A..GA.VKMS.KAS..TF..YWMH  .V
ANO6         ME.HW.F.F..SVTA.VH.Q..P.Q..AE.A..GA.VKMS.KAS...F.RYWMH  .V
ANO4         MGWSW.F.F.LSGTA.VHCQI..KQ...E....GA.VKIS.KAS...F.DY.IN  .V
ANO9         MGWSY.I.F.VATATDVH.Q....QP.AE....GA.VK.S.KAS..TF..YWMH  .V
AN11           MSW.F.F.LSGTA.VH.E....Q...E..R.GA.VKMS.KAS..TF..YVMH  .V
AN12         MEWNWVV.F.LSLTA.VYAQG.M.Q..AE....GA.VK.S.KTS.FTFR.S.IG  .L
ANO8         MEWLWN..F.MA.AQS.QAQI..VQ...E.K..GETVRIS.KAS..TF.TAGIQ  .V
AN10         MNFGFS.IF.VLVLK.VQCE.K.V...G.....GG.LK.S.AAS.FTFS.YAMS  .V

         40             52   53     60          70          82  83    90
          |             ABC          |           |          ABC        |
ANO2     RQFPGNKLEWMGYMS   YSGSTRYNPSLRSRISITRDTSKNQFFLQLKSVTTEDTATYF
ANO1     ..............IN  .D.RNN.....KN................K............Y
ANO3     ..............IN  .D.NNN.....KN................K.N..........Y
ANO7     .....H........IH  .....N.....K.....................N..........Y
ANO5     K.R..QG...I..INP  NT.Y.V..QKFKDKATL.A.K.SSTAYM..S.L.SD.S.V.Y
ANO6     K.R..QG...I..INP  ST.Y.E..QKFKDKATL.A.K.SSTAYM..S.L.S..S.V.Y
ANO4     K.K..QG...I.WIYP  G..NNK..EKFKGKATL.I...SSTVYI..S.L.S....V..
ANO9     ..R..QG...I.EINP  SN.R.N..EKFK.KATLNV.K.SSTAYM.IS.L.S..S.V.Y
AN11     K.K..QG...I..INP  .NDG.K..EKFKGKATL.S.K.SSTAYIE.S.L.S..S.V.Y
AN12     K.K..QS...IAWIYA  GT.G.S..QKFTGKARL.V...SSTAYM.FS.L....S.I.Y
ANO8     QKM..KG.K.I.WINT  R..VPK.AEDFKG.FAFSLE..ASTAY..ISNLRND...A..
AN10     ..T.ERR...VASI.   SGYI.Y.PD.VKG.FT.S..NAR.ILY..MS.LRS....M.Y

          95    100                105  110
           |       ABCDEFGHIJK       |    |
ANO2     CARGWP                  LAYWGQGTQVSVSE
ANO1     ...EDDGYYI              FD.....STLT..S
ANO3     ...EGYGYF               FD......TLT..S
ANO7     ...VIYYYGSSYV          WF.......L.T..A
ANO5     ..YYGSS                YFD......TLT..S
ANO6     ..HYGRS                YFD......TLT..S
ANO4     .V.YGYDG                FG......L.T..A
ANO9     ...R.GSYVGG             F......NM.T..A
AN11     ...FGYYGR            YWYFDV..A..T.T..S
AN12     ...WD.INRG              F.......L.T..A
ANO8     .G.TDYYGST           YYAMD.....SS.T..S
AN10     ...WGHRYDVL              D......S.T..S
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FIG. 1. Deduced amino acid sequences of the *V* regions of the heavy chains of the anti-DNP-SL monoclonal antibodies AN01–AN12.

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                    -20       -10          1          10         20     27
                     |         |           |           |          |      ABCDEF
ANO2                MDFQVQIFSFLLISASVILSRGQIVLTQSPAIMSASPGEKVTMTCSASS
ANO1                ............M.....M............L.................
ANO3                     .......M.....M............L.................
ANO9                ...............I..VM...EN........I...L......S.R...
ANO5                  MRCSLQFLGVLMFWISGVS.D..I..DELSNPVAS..S.SIS.RSTKSLL
ANO6                  MRCSLQFLGVLMFWISGVS.D..I..DELSNPVTS..S.SIS.RSTKSLL
ANO4                  MR.LAELLG.LLFCFLGV.CD.QMN...SSL...L.DTI.I..H..Q
ANO8                  MRF.VQVLG.LLLWISGAQCDVQI....SYLA.....TIIIN.R..K
AN11                  MVFTPQILG.MLFWISA...D........TL.VT..DS.SLS.R..Q
AN12         MHHTSMGIKMES..QV.VFVFLWLSGVD.D..M...HKF..T.V.DR.SI..K..Q
ANO7                   MAW.SLI.SLL.LSSGAIS.A.V..ES. LTT....T..L..RS.N
AN10                   MAW.SLI.SLL.LSSGAIS.A.V..ES. LTT....T..L..RS.T

                30         40         50         60         70         80
                 |          |          |          |          |          |
ANO2             SVYYMYWYQQKPGSSPRLLIYDTSNLASGVPVRFSGSGSGTSYSLTISRMEAEDAA
ANO1             ..S..F......R...KPW..L.........A....................S.......
ANO3             ..S..F......R...KPW.FL.........A....R...............S.......
ANO9             ..N..F.....SDA..K.W..Y.....P...A.........N.......S.AG....
ANO5          YK DGKT.LN.FL.R..Q..Q....LM.TR....SD.........DFT.E...VK...VG
ANO6          YK DGKT.LN.FL.R..Q..Q....LM.TR....SD.........DFT.E...VK...VG
ANO4             NINVWLS.......NI.K....KA...HT...S............FT....SLQP..I.
ANO8             SISK.LA...E...KTNK....SG.T.Q..I.S.........DFT....SL.P..F.
AN11             SVSNNLH.F...SHE......KYA.QSI..I.S.........DFT.S.NSV.T..FG
AN12             DVSTAVA.......Q..K....SA.YRYT...D..T......DFTF...SVQ...L.
ANO7          GAVTTSN.AN.V.E..DHLFTG..GG.N.R.P...A.....LI.DKAA...TGAQT..E.
AN10          GAVT.SNSVK.V.E..DHLFTG..GGSN.R.P...A.....LI.DKAA...AGAQT..E.

               90    95                100    106 109
                |      ABCDEF           |      A   |
ANO2          TYYCQQWSSYPP          ITFGVGTKLEL KRA
ANO1          ..........N..         ....S.....I ...
ANO3          ........N.I..         ....A...... ...
ANO9          .......FT.S.          S...A...... ...
ANO5          V.....LVEF.           L...A...... ...
ANO6          V.....LVEF.           L...A...... ...
ANO4          .......GQ...          L.L.G.....I ...
ANO8          M.....HNE..           Y...G.....I ...
AN11          M.F...SN.W.           F...G.....I ...
AN12          V...H.HY.S.           Y...G.....I ...
ANO7          I.F.AL.Y.NH           LV..G....TVLGQP
AN10          V.F.AL.Y.NH           LV..G.A..TVLGQP
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FIG. 2. Deduced amino acid sequences of the *V* regions of the light chains of the anti-DNP-SL monoclonal antibodies AN01–AN12.